Report of the Haffkine Institute

for the Year 1926

by

LIEUT.-COLONEL F. P. MACKIE, I.M.S.

Director.

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1926

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Mr. R. K. Gokhale, S.M.S.

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Dr. Agnes Murphy, W.M.S.

Dr. B. P. B. Naidu, M.D. (Edin.), M.Hy., D.P.H., D.T.M. (L'pool).

Dr. S. A. Kamat, Ph.D. (Berlin), from 1st February to 31st December 1926.

Medical and Scientific Assistants

Khan Bahadur C. R. Avari, B.M.S. (General Diagnostic Department).

Mr. G. D. Chitre, L.M. & S., B.M.S. (Sprue and Plague Research).

Mr. N. P. Dalal, M.B.B.S., B.M.S. (Plague Vaccine Department) on leave from 1st September 1926.

Jamadar Shamsher Jung, I.M.D. (Plague Research).

Mr. T. G. Akula, S.M.S. (Morbid Histology and Malaria).

Jamadar F. Jasudasan, I.M.D. (Bilharziosis).

Mr. K. H. Bharucha, B.A., B.Sc. (Public Health Chemistry).

Mr. K. J. Tanna, S.M.S., Acting from 21st October 1926 to 31st December 1926.

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„ F. R. Mistri—Head Clerk.
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„ H. J. Parelkar—Engineer.
„ Shaik Mahomedalli—Senior Media Maker.

Junior staff, subordinates and menials numbering 121.

General Review

The output of plague prophylactic during the year has been the largest in the history of the Institute (see chart at page 7a). In addition to the nearly 2½ million doses issued, the end of the year found us with a reserve of about 11 lakhs of doses so that the total manufacture during the year was over 3½ million doses or more than 10 million cubic centimeters.

The vaccine is well received throughout India, and of the Punjab, still the great stronghold of plague, the Director of Public Health writes "its efficacy as a protective measure is now universally recognised and it is consequently a popular measure in almost all districts. No complaints have been recently received in regard to an excessive reaction or in any other respect". This testimony is very satisfactory, and we have received similar statements from other areas. Complaints were made in one district that the reaction after inoculation was insufficient and the vaccine was sent for us for examination. It proved to be of high immunising power but low toxicity due to the ripening or mellowing which takes place with age. It suggests that our efforts to reduce the local toxicity will not necessarily meet with popular approval. The people have learned to expect a good smart reaction and a swollen arm after inoculation and, if this does not occur, to consider themselves aggrieved. The reception of inoculation has undergone a profound change for the better during recent years; for whereas riots were liable to occur when inoculation was pressed, now recently a riot was threatened because the supply of prophylactic ran short.

A great deal of work has been done (of which further details are given in the body of the report) on attempts to improve the quality of the prophylactic, particularly in the preparation of a standard immunising value.

The table on page 9 taken from the experiments of Dr. Naidu shows how variable the immunising value of the vaccine may be.

The work done here by various workers has not succeeded in showing how these variations arise, but the most probable cause lies in the virulence or otherwise of the strains of *B. pestis* used to seed the flasks. There is no way of knowing beforehand whether a strain will be a good antigen or a poor one, its capacities are only revealed later when the vaccine is bottled and tested. As a result we sometimes get "dud" brews and these have to be destroyed and much labour and money lost. All brews are submitted to a stringent test for toxicity and immunising power before being issued, but even so considerable variations are met with.

It was hoped that by buffering the medium and keeping down the alkalinity a better and more constant level of bacterial output would be attained and work on these lines is proceeding.

An enquiry into the action of certain drugs on the course of plague in experimental animals.—Drugs likely to have a high antiseptic value were prepared by Father Caius and Dr. Kamat and were tested by them and by Dr. Naidu on plague infected animals. As was expected the high value of these drugs against plague bacilli in test tubes was not always borne out by their effects on animals.

The common phenols and some of their derivatives—116 drugs in all—were tested *in vitro* for their bactericidal action on *B. pestis*.

The method adopted was the so-called "inhibitory method" and consists in exposing living plague bacilli to the action of the drug for varying periods, 15 minutes to 24 hours, in different dilutions.

The substances examined fall within four main groups :—1, the phenols ; 2, derivatives of phenols ; 3, the phthaleins ; 4, derivatives of fluorescein.

A comparatively high activity was manifested by the following which killed plague bacilli in 15 minutes :—

Quinol	..	1 :	432,000
Mercurochrome, 220 soluble ..	..	1 :	76,800
Catechol	..	1 :	48,000
Alpha-nitroso-betanaphthol ..	..	1 :	25,600
Toluhydroquinone	..	1 :	14,400
2 : 4—diaminophenol ..	..	1 :	12,800
Carvacrol	..	1 :	11,200
2 : 4 : 6—trichlorphenol ..	..	1 :	10,000

As a class the phthalein dyestuffs have a relatively small bactericidal value, and mercurochrome owes its activity to the labile mercury in its molecule.

Experiments on plague transmission.—Owing to the absence of Dr. Chitre on sick leave no further experiments on these lines were carried out. It was felt also that such experiments on the transmissive power of species of fleas are best carried out in the field in areas where plague is spreading rather than under the rather artificial conditions

of the laboratory. The laboratory side of plague transmission has already progressed beyond its application in the field and what is wanted now is a further extension of these principles to practical epidemiology. Work on these lines is being conducted in the United Provinces under the supervision of the Director of Public Health.

Antirabic section.—This section is in its fifth year and is still increasing in scope and in usefulness. The method of bringing antirabic vaccine to the patients rather than the opposite procedure is a great boon and the result is a saving of lives as well as money.

In 84 out of 105 cases the diagnosis of rabies in suspected dogs in the Bombay city was proved by the microscopic test of the brain.

Military Assistant Surgeon H. M. La Frenais to whose tact and energy much of the success of the department is due lays great stress on preliminary cleansing of the wound. He had made it a practice to open up all wounds even those previously cauterized and to apply antiseptic treatment to the wounds and deeper tissues. It is noteworthy that not one of the cases in whom he has done this has subsequently developed rabies and such deaths as have occurred are all amongst those to whom this treatment was not applied.

Schistomiasis and sprue.—Owing to the absence on leave of the two principal investigators on these subjects the work was suspended.

Pharmacological Department.—A full paper on the subject of the therapeutic properties of *Holarrhena antidysenterica*, the investigation on which has occupied Father Caius and Mhaskar since the opening of the department has been completed and sent for publication. Other drugs are being taken up and work on them has been started.

Biochemical Department.—The work of Major Sokhey and his assistants has been directed towards controlling and improving the broth used in the manufacture of plague prophylactic and in certain investigations into the biochemistry of sprue. This, the youngest department of this Institute, is doing good work and promises to be of increased usefulness each succeeding year. Further details are given in the body of the report.

Anæmias of pregnancy.—The enquiry on maternal mortality and particularly on the anæmias of pregnancy was continued throughout the year. In extension of the work carried on by Drs. Margaret Balfour and her assistant Miss Agnes Murphy during 1925, further investigations were carried on in the current year by these ladies assisted by Dr. Miss Mehta and Madame (Dr.) Emmanuelov. An abbreviated report is found in the body of this pamphlet (page 16).

It is beyond question that anæmia is one of the most dangerous complications of pregnancy in this city, the maternal mortality being 43 per cent. and the infantile mortality 53 per cent. The lines on which the investigation has been carried out are in part epidemiological and clinical and in part pathological. The high proportion of urinary infection is noteworthy and further investigations are being carried

out as to their significance. The work for the coming year will include the cause of blood hæmoloysis, the bacteriological condition of the blood, urine and fæces and the chemistry of the blood and of glandular secretions.

Miss Balfour's summary of her investigation is as follows: The statistical enquiry has been completed. Particulars of 11,343 cases of labour are given in a separate report.

The enquiry on anæmia of pregnancy was continued. The number of cases seen in the hospitals lessened considerably during the first part of the year and began to increase again in August and following months. The cases divided themselves into two series—those in which the symptoms were mainly fever and anæmia and those in which in addition diarrhœa was present. The seasonal incidence was connected with the second class only. The maternal mortality of the first series was 14 per cent. and of the second series 43 per cent. The infant death rate on the whole series was 53 per cent.

Many of the patients were well-to-do, living under fair hygienic conditions and able to afford a good diet. 30 per cent. were primiparæ. All communities were affected, but in the present series of cases Mahommedans, Christians and Jews were more largely represented than Hindus.

Of 115 cases in which blood examinations have been made malarial parasites were found in 12 only. In 3 cases the parasites were those of benign tertian, and in 9 cases of malignant tertian. The blood picture was that of a primary anæmia with high colour index and frequent presence of nucleated red cells. In 13 cases in which it was examined bacteriologically it was found to be sterile in 11.

Kahn's precipitation test for syphilis was done in 30 cases of which 15 proved positive and 15 negative, as compared with 43 normal pregnancy controls in which 6 were positive and 37 negative. Both maternal and fœtal mortality were considerably higher in the positive than in the negative cases.

Of 107 cases in which the urine was bacteriologically examined the majority were found to contain organisms. Only 24 per cent. were sterile as compared with 78 per cent. sterile in a series of normal pregnancy cases. These organisms are being investigated further.

Fæces were examined for worm ova in 17 cases in only one of which was hookworm present.

It is hoped during the coming year to investigate the intestinal flora and blood chemistry.

Some observations, clinical and pathological, were made in cases of puerperal sepsis and still birth.

Visit of Professor d'Herelle.—This distinguished scientist paid a visit to the laboratory and worked here from 23rd April 1926 to 12th June 1926 on problems connected with bacteriophage. This fascinating subject is concerned with the destruction of disease-carrying bacteria by still smaller and as yet ultra-visible virus which appears in the tissues of

persons convalescent from disease and is believed to be a living organism. Treatment by bacteriophage has been successful with dysentery in particular but is believed to be applicable to many other bacterial diseases.

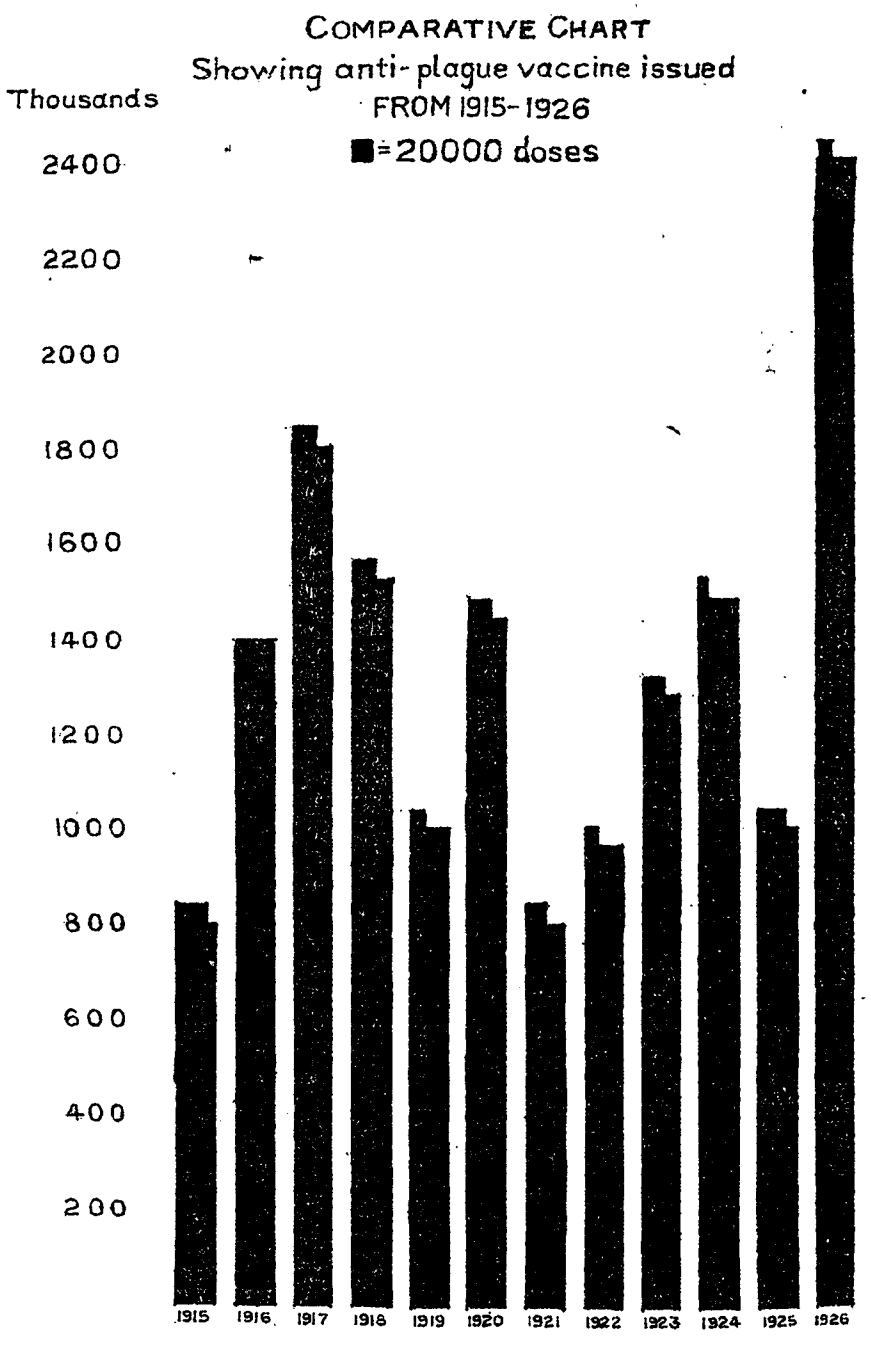
Work on these lines was instituted by Dr. d'Herelle during his visit and has been carried on since. Our experiments are not far enough advanced to justify any conclusions particularly as Dr. d'Herelle has been asked by the Government of India to return to India to resume his work on the treatment of plague, cholera and dysentery by bacteriophage.

The Indian Research Fund Association.—This Association exists for the advancement of research and has been financed by the Government of India. It is administered by a Council, which is advised by a small expert Committee known as the Scientific Advisory Board which includes the Directors of major laboratories and others engaged in medical research. The Haffkine Institute has of recent years been generously assisted with funds by this Association. Both the Pharmacological and the Biochemical Departments owe their inception to the financial assistance of this Association and researches on plague, sprue, bilharziosis have been also financed from this source. A conference of research workers meets every year to review the year's work and to make arrangements for further research during the ensuing year. At this meeting some 40 or 50 research workers from all over India meet together for a week's conference and their recommendations are considered by the Scientific Advisory Board, whose recommendations are put up to the Council of the Association for sanction. At the conference which was held in Calcutta in December 1926 the following schemes were put up by the Director, Haffkine Institute, and have been sanctioned by the Council :—

	Rs.	Rs.
Plague Researches—		
Study of vaccine	38,360	
Transmission enquiry	10,104	
Therapeutic investigation	9,600	
		58,064
Study of Bacteriophage		20,000
Sprue		9,184
Maternal Mortality and Morbidity in Childbirth in India ..		17,270
One-half of recurring charges of the Biochemical Section ..		6,000
Guineaworm Investigation		25,000
Total ..		1,35,518

The sum of Rs. 20,000 for study of the Bacteriophage is the sum set aside for expenses connected with Professor d'Herelle's impending visit.

The Guineaworm investigation is to be carried out in selected villages in the Deccan under the direction of the Director of Public Health, Bombay, and with the assistance of the Haffkine Institute. It has for its object the destruction of the water cyclops, a minute crustacean, which harbours the infective stage of the guineaworm.


COMPARATIVE CHART
Showing anti-plague vaccine issued
FROM 1915-1926
■=20000 doses
Thousands
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926

In addition to these benefits which accrue to Bombay by the action of the Indian Research Fund Association, they have also undertaken to finance a malarial survey in Sind, particularly in the area influenced by the Sukkur Barrage.

The Director has much pleasure in acknowledging the whole-hearted co-operation of the various members of the staff which has contributed so much to the ease of administration and to the satisfactory progress of the Institute.

Manufacture and Distribution of the Plague Prophylactic

From the opening of the laboratory up to end of the year 1926, nearly 28½ million doses of the vaccine have been sent out. The output in each of the last five years has been :—

Year	Number of doses
1922	972,037
1923	1,298,412
1924	1,489,734
1925	1,027,071
1926	2,415,382

The distribution of anti-plague vaccine in India and foreign countries during the last two years is shown below :—

(*Figures represent the number of doses*)

Place or province	1925	1926
Bombay Presidency	111,226	97,251
Madras Presidency	56,736	102,326
Bengal, Bihar and Assam ..	29,031	39,946
United Provinces of Agra and Oudh ..	125,573	228,281
The Punjab	303,292	1,185,880
North-West Frontier Provinces ..	3,501	28,195
Central Provinces and Berar ..	45,269	113,130
Burma	100,012	65,106
Native States in India	241,902	414,789
Countries outside India	10,529	136,228
Delhi Province	....	4,250
Portuguese Territory in India ..	....	....
Total number of doses ..	1,027,071	2,415,382

The following table shows the number of doses of anti-plague vaccine despatched monthly during the past two years :—

Month	1925	1926
January	160,734	176,676
February	179,934	348,948
March	142,612	252,987
April	78,173	280,250
May	12,788	236,235
June	3,225	123,024
July	21,347	13,800
August	56,578	64,722
September	99,890	163,224
October	77,917	216,180
November	92,685	450,356
December	101,188	88,980
Total number of doses ..	1,027,071	2,415,381

Five hundred and seventy-nine thousand eight hundred and six rats alive and dead were sent by the Bombay Municipality to the Institute for examination for Plague. This important work enables the Municipal Health Department to get warning of an outbreak of plague and, as the locality whence each rat came is known, the locality where plague may be expected is foreshadowed.

As we understand these examinations are the largest of the kind in any city in the world, and Bombay in this respect is frequently quoted as a model, the results and their relation to plague in the city are given in the following two tables :—

Examination of Rats for Plague in the City of Bombay, 1926

Month	Alive	Dead	Total	Putrid
January	15,459	38,631	54,090	3,096
February	12,778	32,457	45,235	3,155
March	13,884	34,673	48,557	3,906
April	14,650	36,106	50,756	3,781
May	12,260	34,204	46,464	4,325
June	10,666	31,661	42,327	3,791
July	11,413	34,762	46,175	3,307
August	9,666	28,108	37,774	2,647
September	13,130	29,757	42,887	2,665
October	20,307	37,051	57,358	3,635
November	19,137	30,559	49,696	2,701
December	20,144	38,343	58,487	3,657
Total ..	173,494	406,312	579,806	40,666

Month	Dead rats examined	Plague infected	Rattus Norwegicus	Rattus rattus	Working days
January	27,985	85	76	9	25
February	23,780	121	106	15	23
March	25,925	235	199	36	27
April	23,726	261	221	40	23
May	24,496	198	173	25	26
June	21,028	91	79	12	23
July	26,006	78	67	11	25
August	18,478	79	70	9	24
September	19,881	50	45	5	23
October	26,595	37	24	13	25
November	17,331	13	12	1	20
December	28,267	38	36	2	26
Total ..	283,498	1,286	1,108	178	290

Relation of Deaths from Plague to Plague among Rats, 1926

Period (1926)	Human plague No. of deaths	Total No. of rats examined	Plague infected rats	Percentage	Plague infected Rattus rattus	Plague infected Rattus Norwegicus
1st to 15th January	2	12,976	41	0·3	5	36
16th to 31st January	..	15,009	44	0·3	4	40
1st to 15th February	2	13,051	68	0·5	9	59
16th to 28th February ..	8	10,729	53	0·5	6	47
1st to 15th March	2	12,597	82	0·7	16	66
16th to 31st March	1	13,328	153	1·1	20	133
1st to 15th April	2	11,741	132	1·1	17	115
16th to 30th April	4	11,985	129	1·1	23	106
1st to 15th May	2	13,156	122	0·9	16	106
16th to 31st May	4	11,340	76	0·7	9	67
1st to 15th June	4	11,306	53	0·5	7	46
16th to 30th June	..	9,722	38	0·4	5	33
1st to 15th July	..	14,095	49	0·3	9	40
16th to 31st July	2	11,911	29	0·2	2	27
1st to 15th August	2	11,567	47	0·4	6	41
16th to 31st August	1	6,911	32	0·5	3	29
1st to 15th September	2	9,465	29	0·3	3	26
16th to 30th September ..	2	10,416	21	0·2	2	19
1st to 15th October	2	15,742	23	0·1	10	13
16th to 31st October	..	10,853	14	0·1	3	11
1st to 15th November	..	5,998	3	0·5	..	3
16th to 30th November ..	..	11,333	10	0·9	1	9
1st to 15th December	1	14,883	16	0·1	1	15
16th to 31st December ..	1	13,384	22	0·2	1	21
Total ..	44	283,498	1,286	0·45	178	1,108

Variation in the Potency of different brews of Haffkine's prophylactic

Year	Brew No.	Immunity per cent.
1923	348	33·4
	353	36·9
	372	38·3
	377	45·9
	378	29·0
	380	47·0

Variation in the Potency of different brews of Haffkine's prophylactic—contd.

Year	Brew No.	Immunity per cent.
1924	395	32·0
	405	40·3
	409	52·7
	416	40·0
	427	13·9
	428	36·8
1925	431	31·9
	442	41·7
	443	50·0

1926—62 Brews were examined and out of them 10 gave under 20 per cent. immunity (Bad brews) 21 between 20 per cent.—30 per cent. immunity (Poor brews) 17 between 30 per cent.—40 per cent. immunity (Good brews) and 14 over 40 per cent. immunity (Very good brews).

The per cent. immunity means the percentage number of rats which after inoculation with 0·5 c. c. of the prophylactic, will survive a standard dose of B. Pestis. Nearly 100 per cent. of rats will die of this standard dose if unprotected by a previous dose of vaccine.

Anti-plague Inquiry

During the year, 592 experiments, involving the use of 11,331 rats. 50 guinea-pigs and 49 rabbits, were carried out. These experiments fall under the following heads :—

(*a*) Experiments on the toxicity and potency of Haffkine's plague prophylactic vaccines manufactured in this laboratory for public use,

(*b*) Experiments on the prophylactic and therapeutic value of bacteriophage in experimental animals,

(*c*) Experiments on the standardisation of methods for estimating the potency of plague vaccines,

(*d*) Experiments on the antiseptic value of certain of the phenol series,

(*e*) Experiments on the natural immunity of Bombay rats against experimental infection with plague, and

(*f*) Experiments on the production of a high agglutinating and therapeutic anti-plague serum.

1. Experiments carried out on the nutrient broth now used for the culture of plague bacilli and its hydrogen-ion concentration seem to indicate that the existing method of adjusting the reaction of the culture medium in this laboratory satisfies the requirement of the hydrogen-ion concentration for the optimum growth of plague bacilli.

2. Experiments on the production of alkalinity of *B. pestis* in broth and the effect of this alkalinity on the toxicity and potency of plague vaccines, seem to indicate—

(*a*) that during the growth of plague bacilli the medium becomes alkaline,

(*b*) that this alkalinity of the medium steadily increases in amount from week to week reaching a maximum point between the fifth and the eleventh week of inoculation,

(*c*) that this is followed by a series of small rises and falls in the amount of alkalinity, throughout the period of incubation extending to 176 days.

Even though the broth culture of plague bacilli is incubated for a period of 176 days, the organisms are still viable. The amount of alkalinity present in the vaccine has apparently no influence either on its toxicity or its potency.

3. Seventy-four brews of the plague prophylactic vaccine have been tested on rats for their potency, and in cases where some vaccines gave a low potency the experiments were repeated with similar results. It would, therefore, appear that this method of testing the potency of the vaccines offers a means of standardising the vaccines; in view of the accidental deaths occurring in rats during experimentation as a result of handling, experiments are now in progress to standardise methods for the estimation of potency on guinea-pigs.

4. Experiments on the toxicity and potency of plague vaccines grown in other culture media, such as trypsinbroth, casein-broth, papaya-digest broth, seem to indicate that a culture of plague bacilli on the casein-broth yields a vaccine of slightly higher potency.

5. Experiments on the standardisation of the test dose of plague for estimating the therapeutic value of drugs in guinea-pigs and rabbits are in progress; from the experiments so far carried out, it would appear that even when the test dose now employed is diluted by several millions, produces death in experimental animals within five to seven days of infection.

6. Experiments on the antiseptic value of drugs against plague bacilli have been carried throughout the year, and the results are embodied in a paper which will soon appear in the Journal of Indian Medical Research.

7. In view of the results obtained on the immunity of Bombay rats to experimental infection with plague in 1924, experiments were throughout the year carried out on Bombay rats (Mus rattus) to estimate their resistance to experimental infection with plague at fortnightly intervals; these results seem to indicate that the immunity of Bombay rats varies considerably during the course of the year, and that this variation has some relation to the seasonal prevalence of the disease in the city. These experiments are being continued.

8. Some experiments were carried out for the isolation of a potent bacteriophage from infected rats on the lines suggested by Dr. d'Herelle. These have not yielded a potent bacteriophage.

9. At the instance of the Government of India, Dr. Naidu, Dr. Avari and Dr. Marras of Port Said were deputed to study the therapeutic value of d'Herelle's pestiphage in human cases of plague at Hyderabad (Deccan) and at Agra during the months of March, April and May last. The results showed that neither the course of the disease nor the mortality from it was influenced by the administration of the bacteriophage, either subcutaneously into the bubo or intravenously, or by a combination of both methods.

Transmission of Plague by Fleas

Investigation into the transmission of plague by the fleas *Xenopsylla cheopis* and *Xenopsylla astia* was continued by Dr. Chitre till his departure on leave early in April. As mentioned in last year's annual report the rats used for these experiments were completely rid of their fleas by giving them a momentary dip in petrol and this method was found to be quite satisfactory for this purpose. Out of an aggregate of twelve experiments carried out during this period, three had to be discontinued for want of fleas, eight had to be cancelled for various reasons which interfered with the accuracy of the experiment, and in the remaining one, in which the result was comparable, successful transmission did not take place either with *cheopis* or with *astia* although the " A " rats had died of plague.

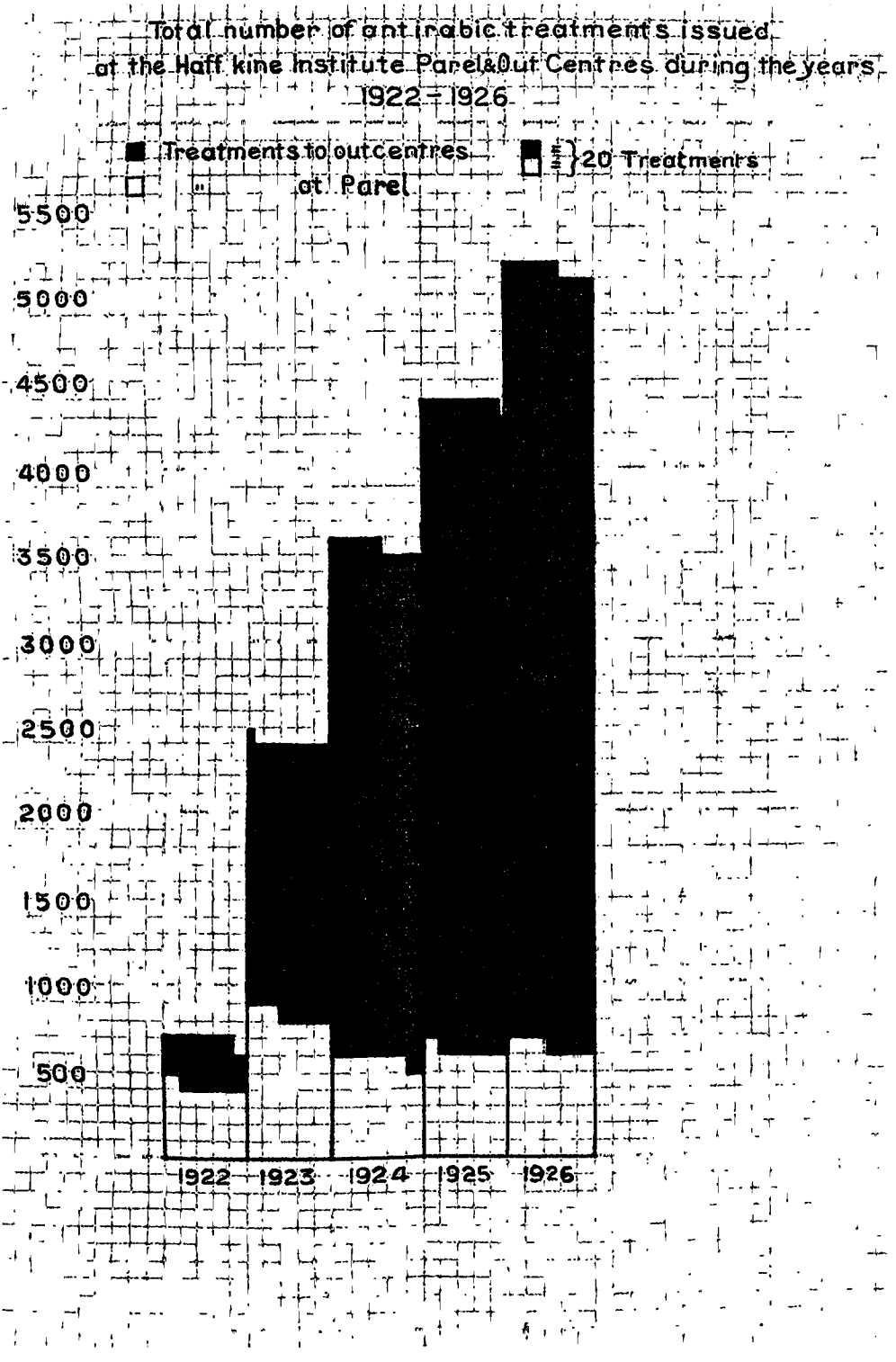
Rat Destruction

Two mud huts were built inside two of the animal house godowns during the month of October 1925. In both these huts a certain number of marked male and female *Rattus rattus* were let loose and they were allowed to breed and burrow during subsequent months. About the middle of December of the year under review a single intensive baiting campaign was carried out in mud hut No. 1 with improved Barium carbonate baits, in the presence of plenty of mixture of grains, green vegetables and water. The approximate ratio between the aggregate weight of the baits and the aggregate weight of the food supply simultaneously available inside the godown was about 1 to 3. The mud hut was pulled down after this single intensive baiting and it was ascertained that we had succeeded in killing approximately 42 per cent. of the rats infesting the hut.

Anti-rabic Section

The anti-rabic section in the fifth year of its existence still continues to develop. During the year 1259 persons applied for anti-rabic treatment in this Institute, out of whom 648 persons underwent the full course of treatment, 120 persons discontinued treatment, and 491 persons were given advice but no treatment.

The statistics of the 648 persons treated are being shown in detail in the attached tables. Of these one person died within less than 15 days after the completion of treatment and four died more than 15 days after the completion of treatment. The latter classed as failures gives a failure rate of 0·6 per cent. Twenty-two persons out of this number were treated intravenously and there was one death among them.


Total number of antirabic treatments issued
at the Haffkine Institute Parel&Out Centres during the years
1922 - 1926
Treatments to out centres
at Parel
20 Treatments
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
1922
1923
1924
1925
1926

Treatment to out centres—4,385 treatments were issued to the various (31) centres of which 13 are under the control of the Surgeon General with the Government of Bombay, and the remaining 18 are in the Central Provinces, United Provinces and Native States, Military Department, the Railways and Goa. Out of these only 4,148 underwent the full course of treatment.

One hundred and fourteen treatments were issued to Veterinary Surgeons for treating animals bitten by rabid animals.

The brains of 105 dogs in Bombay City or in the adjoining suburb of Bandra were received for examination. Of these 2 were received in a condition unfit for examination, in 19 no evidence of rabies was found, while Negri bodies were present in 84.

One hundred and twelve dogs' brains were received from out-stations. Of these 16 were unfit for examination, in 27 no evidence of rabies was found, and 69 were proved to be rabid.

During the year two drugs, Mercurochrome and Bayer 205, were tried for their curative effect in fixed virus rabbits. The results obtained were negative.

Two indigenous drugs sent by a local Vaidya claiming to cure Hydrophobia in all its stages were tried, with negative results.

Pharmacological Department

I.—Indigenous Drugs—

Holarrhena anti-dysenterica.—A general study of this plant was concluded in the course of the year and the results embodied in a paper which has been sent for publication. The following conclusions were arrived at: (1) it is neither anthelmintic, nor stimulant, nor astringent, nor styptic; (2) it is a bitter which increases appetite and digestive power; (3) its anti-dysenteric value compares favourably with that of any of the medicines now in vogue; (4) its anti-diarrhœal properties are not dependent on one chemical constituent in particular, but—if we except cellulose and lignin—on the entire seed or bark; (5) it affords a safe, cheap, reliable, and easily administered drug for the treatment of diarrhœic troubles.

Wrightia tinctoria.—The anti-diarrhœal effects of its seeds agree with those of *H. antidysenterica* both as regards nature and intensity. On the other hand the bark is markedly inferior.

Wrightia tomentosa.—The bark is inert in the treatment of dysentery.

Alstonia scholaris.—The bark is but slightly inferior to that of *H. anti-dysenterica in the treatment of dysentery.*

Garcinia mangostana.—Fifty-five patients suffering from diarrhœa or dysentery were treated with the powdered rind of the fruit, and 36 were benefited by the treatment.

Butea frondosa.—a. The work with the seeds has been temporarily suspended. The good results we obtained with an alcoholic extract could not be confirmed and we have not been able to identify as yet

any active principle responsible for the action of the seeds on ascarids after elimination of the inert fixed oil.

b. The leaves are therapeutically inert.

Gymnema sylvestre.—The preliminary chemical and pharmacological examination has been completed. The powdered leaves have been administered to six cases of glycosuria and they appeared to be effective in two cases.

Nyctanthes arbor-tristis.—The powdered leaves were administered to 31 malarial patients of whom 13 showed no parasites in the blood on discharge and had no temperature. Six required to be treated with quinine as they proved refractory to *Nyctanthes* treatment. In 7 the temperature remained normal after treatment, but crescents were found in the blood. Five left hospital with a normal temperature, and blood films were not available for examination. The pharmacological examination is being carried out.

Terminalia arjuna and T. tomentosa.—Work is in progress.

II. Helminthiasis.—

Since August 1924 we have examined 3,460 prisoners for the presence of helminthic infection. Hookworm infection was found to be present in 45·1 per cent., roundworm infection in 24·4 per cent., and whipworm infection in 63·9 per cent. of cases.

Biochemical Unit

During the year the Unit has been engaged on investigations connected with Haffkine's Plague Prophylactic and on Sprue enquiry.

I. Haffkine's Prophylactic.—(1) A new method for the measurement of the growth of *B. pestis* in liquid medium: As difficulties were encountered with the opacity method and the method of counting colonies an effort was made to devise an alternative method. With this object in view CO_2 given out by the organisms during growth was measured and compared with the weight of the organisms grown. It was found that there was a definite co-relation between the weight of organisms and the amount of CO_2 given out: 1 cc. of CO_2 produced equalled $0{\cdot}41 \pm 0{\cdot}05$ mg. of dry weight of organisms.

(2) The effect of Amino-acids on growth of *B. pestis*: To reduce the salt content and also possibly to enrich the medium glycocoll was used to buffer laboratory broth. It was found that 2·5 per cent. concentration of this amino-acid prevented the growth of the organism altogether. While 1 per cent. of amino-acid—the least quantity we could use profitably to buffer—gave poor growth. This suggested an enquiry into the presence or production of amino-acids in broth as a possible hindering factor. The amino-acid content of the laboratory broth was found to be about 0·15 to 0·20 per cent. and did not rise above 0·5 per cent. during several weeks growth. This amount of amino-acid was not found to be an injurious factor.

(3) Isolation of the antigenic factor from Haffkine's prophylactic: Filtrates obtained by filtering unheated Haffkine's prophylactic through

Pasteur-Chamberland filters were found to possess almost as high immunising value as the original prophylactic. Original broth and this filtrate were subjected to analysis, and it was found that a heat coagulable protein—most probably an albumin—was present in the filtrate which was not present in the original broth. This substance when precipitated with the proteoses present in the broth, and redissolved in slightly alkaline saline gave a high immunity figure. Further work on this substance is being carried out.

II. Sprue.—On the return of the Director from leave the sprue enquiry was re-opened and biochemical investigations on the original plan were re-started. In addition fresh lines of attack, as very kindly suggested by Dr. Fairley from his extensive knowledge of the literature, were adopted.

(1) Liver Function Tests.—Tests employed (*a*) Lævulose tolerance; (*b*) Brom-sulphlein Dye, (*c*) Non-protein Nitrogen and Urea Nitrogen of blood, (*d*) Van den Bergh test.

	Cases examined
Sprue chronic	6
Sprue early	4
Sprue doubtful	1
Pernicious anæmia	2
Post partum anæmia— a. severe	1
b. moderate	1
Odd cases	6

Results.

(*a*) Lævulose Tolerance Test.

	Abnormal	Normal
Sprue chronic	4	2
Sprue early	1	3
Sprue doubtful	0	1
Pernicious anæmia	2	0
Post Partum anæmia severe	1	0
Moderate	0	1

(*b*) Brom-Sulphalein Dye Test.

At the end of half an hour 5 per cent. of the dye had been retained in blood in three cases only, sprue chronic 2, P. A. 1, so it may be said this test did not show any abnormality of the liver. This test it seems can detect only gross lesions of the liver; this point to be tested as opportunity occurs.

(*c*) Non-protein N and Urea N of blood did not disclose any abnormality in any of the above cases.

(*d*) Van den Bergh test.

Direct reaction—invariably absent or delayed faint. Indirect reaction—positive, going up to four units. The reaction is qualitatively the same in pernicious anæmia, and shows extra hepatic hæmolysis.

(2) Pancreatic efficiency test.

Duodenal contents of two cases of sprue chronic (both cases included in the previous series, and of 4 or 5 years' duration) showed diastase, trypsin, and lipase normally active. A case of pernicious anæmia gave the same result.

(3) Calcium content of blood.

Total varied between 10·8 and 10·1 mgs. per cent.

Ionic (Vine's) varied between 9·8 and 8·6 mgs. per cent.

(4) Fat in fæces.

Saxon's wet method has been employed in place of Cammidge's it avoids some of the obvious shortcomings of the latter method. The cases studied so far do not show any marked variations from the normal, except in increased total content of fat.

(5) Glucose tolerance test.

Carried out in three chronic cases, no abnormality.

Enquiry on Maternal Mortality and Morbidity in Childbirth

The enquiry on anæmia of pregnancy has been continued by Dr. Murphy assisted by Dr. Mehta. Up to the present 138 cases have been followed up in Bombay hospitals and in their own homes.

(1) One hundred and fifteen blood examinations have been made. Malarial parasites were found in 12 cases, of which 3 were p. vivax and 9 were p. falciform. The blood picture presented few variations and the following is the average of 25 consecutive examinations :—

Erythrocytes per c. m. m. ..	1,210,000	
Hæmoglobin	33	per cent.
Colour index	1·6	
Leucocytes per c. m. m.	8,416	
Polymorphs	57·1	per cent.
Lymphocytes	38·6	per cent.
Mononuclears	3·5	per cent.
Eosinophiles	0·88	per cent.
Poikilocytosis was present in ..	13	
Anisocytosis and Polychromatophilia in	24	
Achromia in	23	
Basophilic degeneration in	9	
Malaria parasites in	1	
Normoblasts in	16	
Megaloblasts in	11	

The red cells varied from 500,000 to 2,830,000 and
The leucocytes from 3,200 to 32,000 per c.m.m.
Platelets absent or scanty.

In 13 cases in which the blood was cultured it was found to be sterile in 11. In one case streptococci (non hæmolytic) were present and in one case gram negative bacilli.

Kahn's precipitation test for syphilis was done in 30 cases of which 15 proved positive and 15 negative, as compared with 43 normal pregnancy controls in which 6 were positive and 37 negative.

Blood grouping of mother and child was investigated in 36 cases. The maternal and fœtal blood was in the same group in 10 cases and in different groups in 26 cases as compared with 17 normal pregnancy controls where the blood was in the same groups in 12 cases and in different groups in 5 cases only.

	Different groups	Same groups
Anæmic cases ..	72·2 per cent.	27·8 per cent.
Normal cases ..	29·4 „	70·6 „

(2) Urine :—Of 107 cases examined for albumen 52 were positive, and 55 negative. Of 150 cases bacteriologically examined 37 were sterile, in the remainder the following organisms were present :—

B. alkaligenes	23 cases.
B. pyocyaneus	18 „
Streptococci	7 „
Staphylococci	5 „
Coliform organisms	32 „
Gram negative organisms not further identified:	28 „

The urine was examined in 41 cases of normal pregnancy and of these 32 were found to be sterile. Of the remainder 6 contained *B. alkaligenes*, 2 contained gram negative organisms, and 1 contained *B. ærogenes*. Vaccines were prepared and administered in 4 cases. Three patients improved the fourth died the day after the first dose. She was very seriously ill.

(3) Fæces were examined for worm ova in 17 cases. In 5 roundworm were present, in one whipworm and in one hookworm. The remainder were negative.

In addition to the above a number of examinations have been made in cases of doubtful pregnancy anæmia, non-pregnant anæmia and fever in pregnancy.

Comparing these results with the clinical notes it appears that the cases with malarial parasite in the blood were less liable to digestive symptoms and the maternal mortality was less. Three of those with malignant malaria died, but they had not been treated with quinine.

No deaths occurred among those treated with quinine. Probably more cases were due to malaria but the plasmodium was not found in the peripheral blood. It is intended to search for it in the placenta.

Of the remaining cases, the maternal and fœtal mortality was considerably higher in those positive to Kahn's test. In these the maternal mortality was 60 per cent. and the fœtal mortality 85 per cent. as compared with maternal mortality of 43 per cent. and a fœtal mortality of 52·8 per cent. for the whole series (138 cases).

It is hoped during the coming year to examine the intestinal flora and investigate the blood chemistry.

The intrauterine discharge was examined in 23 cases of puerperal sepsis and the following organisms were found :—11 coliform, 9 streptococci (non hæmolytic), 1 staphylococci, 2 were sterile.

Sixty-one placentæ were examined macroscopically in cases of stillbirth. In 33 of these cases the maternal blood was examined by Kahn's test for syphilis and 21 were found to be positive and 12 negative, that is 63·7 per cent. were positive as compared with 13·4 per cent. of 67 normal pregnancy controls.

Chemical Department

Eighteen samples of water were examined bacteriologically with a view to determine : (1) the total number of ærobic bacteria growing well on nutrient agar per cubic centimeter of water, and (2) the minimum quantity of water fermenting MacConkey's neutral-red-lactose-bile-salt-broth with the production of acid and gas when incubated at 37°C. for 24 hours. The results thus obtained are shown in the following table :—

Source of water	Number of samples	Aerobic bacteria per c.c. of water varied from	Minimum quantity of water fermenting MacConkey's broth varied from
Tanks	2	7 to 3300	10 c.c. to 0·1 c.c.
Wells	3	1500 to 17000	0·1 c.c. to 0·001 c.c.
Lakes	2	3000 to 8000	10 c.c. to 1·0 c.c.
Filter Chamber	1	3200	0·1 c.c.
No. 4	1	9600	1·0 c.c.
Pipes*	9	70 to 450	1·0 c.c. to 0·01 c.c.

* In one sample ærobic bacteria were 4000 per c.c.

There was one sample of milk which on a bacteriological examination showed 272,000,000 ærobic bacteria per cubic centimeter and 0·0000001 c.c. as the smallest quantity of milk which contained bacteria fermenting MacConkey's broth.

One hundred and twenty-two samples of water were analysed chemically.

Table showing the analysis of the malignant tumours and their distribution in the body

Organ	Carcinoma	Sarcoma	Organ	Carcinoma	Sarcoma
Anus	3	..	Lip	1	..
Arm	..	1	Maxilla ..	..	1
Aorta	..	1	Nose	3	..
Abdomen ..	..	1	Neck	2	..
Breast	9	..	Omentum ..	..	1
Bowel	1	..	Orbit	2	.. (1 perithelioma,)
Buttock ..	..	1	Placenta ..	1	..
Cervix	2	..	Penis	2	..
Chest	..	1	Parotid ..	..	2
Cheek	..	1	Rectum ..	1	..
Eye	4	1	Scrotum ..	..	1
Foot	3	1	Shoulder ..	..	1
Gland	3	..	Thigh	1	2
Hand	2	..	Tonsil	1	..
Jaw	1	..	Testis	..	2
Kidney ..	1	1	Tibia	..	1
Liver	2	..	Uterus ..	1	2
Leg	1	..	Vagina ..	..	1
			Total ..	47	23 = 70

Clinical Diagnosis, Vaccines, etc.

(1) Wassermann examination (by Nogouchi's modification). The Sachs-Georgi method of diagnosis was carried out side by side with the Wassermann reaction.	Positive ..	462	
	Negative ..	637	
			1,009
Sachs-Georgi reaction ..	Positive ..	419	
	Negative ..	680	
			1,099
Formal-Gel	Positive ..	27	
	Negative ..	14	
			41
(2) Other routine diagnostic examinations			3,181
(3) Autogenous vaccine prepared for cases			293
(4) Pathological tissues for diagnosis—			
(*a*) Non-malignant		81	
(*b*) Malignant		70	(for details see table above.)
(*c*) Granulomata		34	
(*d*) Other pathological conditions . ..		125	
			310

(5) Vaccines prepared (other than Haffkine's prophylactic and anti-rabic vaccine)—

Mixed T.A.B. vaccine	4,400 c.cs.
Cholera vaccine	4,800 c.cs.

(6) Bacterial suspensions prepared for agglutination tests—

Typhoid	770 c.cs.
Paratyphoid A	1,200 c.cs.
„ B	990 c.cs.
„ C	450 c.cs.

(7) Stock cultures of bacteria—

Three hundred and seventy-five stock cultures were sub-cultured at least once a month.

Thirteen special cultures of B. pneumococci were sub-cultured on special media every four days.

(8) Bacterial suspensions and cultures supplied on indent—

Vaccines—

Mixed T.A.B.	1,700 doses.
Cholera	1,175 „

Dead Emulsions—

Typhoid	134 c.cs.
Paratyphoid A	95 „
„ B	93 „
M. melitensis	4 „
High Titre serum T.A.B.	1 c.c.

F. P. MACKIE,
Lieut.-Col., I.M.S.,
Director, Haffkine Institute.

Parel, Bombay, 4th May 1927.

APPENDIX

TABLE I

Statistics for persons treated at Parel, 1922-1926

Year	Total treated	Failures		Deaths		Indians
		Total No.	Percentage	Total No.	Percentage	Treated
1922	420	4	0·93	4	0·93	365
1923	845	9	1·06	10	1·18	730
1924	587	7	1·19	10	1·70	525
1925	625	5	0·8	6	0·96	583
1926	648	4	0·6	5	0·76	588

Year	Indians—*contd.*		Europeans			Cases extracted Advice cases and incomplete treatment
	Deaths	Percentage	Treated	Deaths	Percentage	
1922	4	1·09	64	..	..	212
1923	10	1·37	115	..	..	523
1924	9	1·71	62	1	1·61	513
1925	6	1·02	42	..	..	355
1926	5	0·87	00	..	..	611

TABLE II

Classification of patients by probability of rabies in the biting animals

*=Fatal cases

Class	Sub-class I Bitten on head or face	Sub-class II Bitten through bare skin any where except on the head or face	Sub-class III Bitten through clothing	Sub-class IV "Licks" on any part of the body	Total	Percentage of deaths
Part I						
Class A.—Bitten or licked by animals proved rabid.	5	2* 81	23	14	123	1·6%
Class B.—Bitten or licked by animals certified rabid.	3	1* 91	43	1	138	0·7%
Class C.—Bitten or licked by animals suspected rabid.	18	2* 251	112	6	387	0·5
Total ..	26	423 5*	178	21	648	0·771%
Part II			*Europeans*			
Class A.—Bitten or licked by animals proved rabid.	2	18	6	6	32	..
Class B.—Bitten or licked by animals certified rabid.	..	8	4	1	13	..
Class C.—Bitten or licked by animals suspected rabid.	1	2	9	3	15	..
Total ..	3	28	19	10	60	..
Part III			*Indians*			
Class A.—Bitten or licked by animals proved rabid.	3	2* 63	17	8	91	2·2%
Class B.—Bitten or licked by animals certified rabid.	3	1* 83	39	..	125	0·8%
Class C.—Bitten or licked by animals suspected rabid.	17	2* 249	103	3	372	0·56%
Total ..	23	5* 395	159	11	588	0·85%

TABLE III

Cases developing Hydrophobia within 15 days of completion of treatment

Case No.	Sex	Age in years	Animal	Number and position of the bite and total No. of deep bites given (last in brackets)	Whether on bare skin or through clothing	Whether cauterized or not
784	Male ..	10	Dog ..	4, Arm ..	Bare ..	Cauterized ..

Date of commencing treatment	Number of days late	Duration of illness	Interval between bite and death	Remarks
2 Sept. 1926 ..	....	3 days ..	29 days.	

TABLE IV

Cases developing Hydrophobia later than 15 days after completion of treatment "Failures"

Case No.	Sex	Age in years	Animal	Number and position of the bite and total No. of deep bites	Whether on bare skin or through clothing	Whether cauterized or not
193	Male ..	6	Dog ..	6, leg ..	Bare ..	Cauterized ..
388	Do. ..	55	Do. ..	6, Trunk and arms.	Do. ..	Do. ..
478	Do. ..	10	Do. ..	1, Leg ..	Do.	Uncauterised ..
585	Female ..	5	Jackal ..	10 Leg. ..	Do. ..	Uncauterised ..

Date of commencing treatment	Number of days late	Duration of illness	Interval between bite and death	Remarks
5th Mar. 1926 ..	1 day ..	2 days ..	30 days.	
1st May 1926 ..	3 days ..	1 day ..	30 ,,	
20th May 1926 ..	2 ,,	1 ,, ..	66 ,,	
16th June 1926 ..	3 ,, ..	2 days ..	34 ,,	

TABLE V

Showing the animals which bit, scratched or licked the patients

Species of animal	Number of persons bitten or scratched or licked	No. of deaths
Dog	608	4
Jackal	24	1
Human beings	1	
Horse	3	
Cat	5	
Monkey	7	
Total ..	648	

TABLE VI

Showing the number of persons who came for treatment each month during the year 1926

Month	Discontinued treatment	Europeans		Indians		Deaths
		Treated cases	Advice cases	Treated cases	Advice cases	
January	5	4	2	44	22	..
February	7	9	3	57	18	..
March	18	5	8	59	34	..
April	12	4	2	33	38	..
May	12	6	10	56	62	2
June	7	7	4	45	45	..
July	10	3	..	34	26	..
August	8	4	4	29	26	..
September	4	3	4	49	34	2
October	12	8	1	70	35	1
November	15	3	6	65	53	..
December	10	4	7	47	47	..
Total ..	120	60	51	588	440	5

TABLE VII

Total number of patients treated at Parel according to provinces from which they came

Province	Europeans	Indians	Total
Bombay City	46	535	581
Bombay Presidency	8	42	50
Central Provinces	..	1	1
Rajputana	3	4	7
Goa	1	1	2
United Provinces	1	2	3
Native States	1	3	4
Total ..	60	588	648

Further statistics if required will be supplied on application to the Director, Haffkine Institute, Parel, Bombay.

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M O P I—4

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